Identification of Synthetic Inducers and Inhibitors of the Quorum-Sensing Regulator LasR in \textit{Pseudomonas aeruginosa} by High-Throughput Screening

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Abstract

We report the screening of 16,000 synthetic compounds for induction and inhibition of quorum sensing in a *Pseudomonas putida* AHL sensor strain engineered with the LasR transcriptional activator. LasR controls virulence gene expression in the opportunistic pathogen *Pseudomonas aeruginosa* and is of significant interest as a therapeutic target. Nine compounds that inhibit and 14 compounds that induce LasR activity were identified in our high-throughput screen.

Introduction

Quorum sensing (QS) is a mode of microbial communication that is essential to controlling activities associated with pathogenesis, symbiosis, and interactions of key ecological importance (6, 18). Many Proteobacteria exhibit QS behavior through the exchange of N-acylated L-homoserine lactone (AHL) signaling molecules in a cell-density dependent manner to regulate gene expression (22). Archetypal AHL-mediated QS systems consist of LuxR-type transcriptional regulators and LuxI-type synthases that produce AHLs. Members of the LuxR-family interact with a cognate AHL in a concentration-dependent manner to regulate the expression of genes.

The role of QS in *P. aeruginosa* pathogenicity has been demonstrated by mutant analysis in multiple host-pathogen systems (9, 14). The linkage between QS and the production of virulence factors in *P. aeruginosa* predicts that inhibition of AHL-mediated gene expression could render these bacteria nonpathogenic (8). Studies on the rational
design of QS inhibitors have focused on modifying the basic structure of the AHL (7, 13). High-throughput screening (HTS) of small molecule libraries is another approach for the identification of compounds that have novel chemical scaffolds for both the development of chemical probes and eventual therapeutic drug design. Previous HTS of a sizable corporate compound library identified two inhibitors structurally similar to AHLs and one non-native inducer of LasR (11, 12). The inducer, triphenyl derivative TP-1, was structurally unrelated to AHLs and exhibited higher stability, and thus more prolonged activity, than $N$-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL). This result serves to validate the HTS approach for the identification of non-native modulators of QS. Here, we report complimentary HTS efforts for the identification of novel small molecules that are modulators of the LasR QS circuit and structurally dissimilar to native AHLs.

Small molecule modulators of QS are frequently identified using bacterial reporter strains (1, 17). We used *P. putida* strain F117 pKRC12, an AHL-deficient strain, that has been engineered to produce GFP upon activation of LasR by $N$-(3-oxo-decanoyl)-L-homoserine lactone (3-oxo-C10-HSL) and 3-oxo-C12-HSL (19, 20). Our HTS approach used 3-oxo-C10-HSL as the inducer of LasR-mediated signaling to identify compounds that either inhibit or induce GFP expression. We speculated that 3-oxo-C10-HSL binds LasR with a weaker affinity than 3-oxo-C12-HSL, which would enable the simultaneous identification of compounds that either reduce or increase the activity of LasR. Due to its ready availability and relative structural diversity, we selected the 16,000-member Chembridge DIVERSet (ChemBridge Corporation, San Diego, CA) collection for our HTS. This library was screened at a final compound concentration of
3.3 μM. Bacterial cells were added to the compounds approximately 30 min prior to the addition of 3-oxo-C10-HSL at a final concentration of 100 nM, the EC$_{50}$ of the compound in the $P$. putida F117 pKRC12 AHL reporter strain. Additional information regarding reporter assays and bacterial strains are provided in supplementary materials. Compounds that reduced or increased the fluorescence by a factor greater than or equal to three standard deviations from the mean values for the control, 3-oxo-C10-HSL, were chosen for further analysis and subjected to additional screening.

We identified nine antagonists (X2-X10) and 14 agonists (A1-A14) of LasR-mediated QS (Fig. 1). Antagonists designated X5, X6, and X7 share structural similarities and contain a thiocarbamate linked to an aniline ring through an amide bond with various functional group substitutions. In addition, some of the agonists share structural similarities and comprise two closely related families of compounds. Agonists A7 and A11 both contain two amide bonds that constitute a tri-phenyl backbone that displays varied functionality on the phenyl rings. Agonists A2, A3, and A4 share a scaffold with a furanyl ring, cyano group, and amide bond. Compounds A1 and A5 are also structurally similar, with the exception of the furanyl group from A1 and lack of an amide bond in A5. Overall, the identification of antagonists and agonists that share structural similarities is a strong indicator that the HTS was robust and demanded some degree of structural specificity. The antagonists identified by HTS differed structurally from previously described antagonists resembling 3-oxo-C12-HSL (Fig. 1).

To evaluate the activity of antagonists against the natural AHL ligand of LasR, 3-oxo-C12-HSL was used as the inducer in antagonism assays (Fig. 2). Three of the antagonists inhibited biosensor activity by more than 50% at a concentration of 20 μM.
against 3-oxo-C12-HSL (at 50 nM). Notably, bacterial growth was not affected by the
addition of the antagonists at the concentration tested (data not shown). When 3-oxo-
C12-HSL concentration was increased 20-fold, inhibition was not detected, suggesting
competitive interaction between the antagonists and 3-oxo-C12-HSL for LasR binding
(Supplementary Fig. 1). Loss of inhibitory activity at the higher concentration suggests
that the antagonists are outcompeted when 3-oxo-C12-HSL saturates the receptor, and
provides further evidence that the antagonists interact specifically with LasR.

To further characterize the agonists, we measured activity at various
concentrations. At a final concentration of 10 μM, all 14 of the agonists activated
expression of the biosensor in the absence of exogenous AHL (Fig. 3). Moreover, the
activity of agonists A2 and A9 was comparable to natural ligand 3-oxo-C12-HSL at 10X
the concentration (500 nM). Interestingly, these two compounds were structurally less
similar than other agonists identified in this study (such as A1-A5, and A7 and A11).

The specificities of the antagonists and agonists for LasR and additional LuxR-
type proteins were evaluated in biosensor strains with different LuxR-type proteins and
host backgrounds. The agonists were active and exhibited the same relative trend of
activity in P. putida F117 pKRC12 and E. coli DH10B pKRC12, which both contain the
LasR transcriptional regulator. These results suggest that agonist activity is independent
of host background. Agonists were not active in the modified ΔlasR reporter strain, P.
putida F117 pKRC12ΔlasR, indicating that the agonists interact directly with LasR. As
expected, neither the agonists nor the antagonists were active in AHL reporters that detect
short chain AHLs with different LuxR-type transcriptional regulators, such as LuxR,
TraR, and CviR, suggesting that these non-native ligands are specific for LasR.
To understand how non-native agonists and antagonists could be accommodated by the ligand binding site, we performed computational docking studies (see supplementary materials) using the reported X-ray crystal structure of the N-terminal domain of LasR (PDB code: 2UV0) (3). Modeling of the thiocarbamate antagonists showed overall agreement between activity and the ability to mimic the natural ligand. In contrast, the most active agonist acts on LasR either by binding to an allosteric site of LasR or by significantly changing the conformation of the binding pocket of the natural ligand and may provide a strategy for design of new compounds that modulate the activity of LasR through alternative interactions.

In summary, we have identified nine non-native antagonists and 14 non-native agonists of LasR-mediated gene regulation via HTS. Notably, these compounds are structurally distinct from other known agonists and antagonists, and hold promise as new scaffolds for the development of chemical tools to probe the mechanisms of QS in *P. aeruginosa*, and could, with further development, provide a pathway for the design of novel anti-virulence agents.

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**Figure Legends**

**FIG. 1.** Structures of the antagonists (X2–X10) and agonists (A1–A14) identified in this study. Structure of the native ligand of LasR, N-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL).

**FIG. 2.** Antagonism screening data. Activity of *P. putida* F117 pKRC12 after 4 h when induced by 50 nM 3-oxo-C12-HSL and challenged with 20 μM antagonists X2–X10. Values reported are the mean specific fluorescence activity of each compound normalized as a percentage of the control for four replicates. Error bars indicate the standard deviation.

**FIG. 3.** Agonism screening data. Activity of compounds A1–A14 compared to 3-oxo-12-HSL at final concentrations of 0.5 μM (■), 1 μM (□), and 10 μM (▲) to determine their ability to activate LasR-regulated gene expression in *P. putida* F117 pKRC12. 3-oxo-C12-HSL (50 nM) was included for comparison of agonist activities to the natural ligand. DMSO was included as a negative control. Specific fluorescence was determined after 4 h of growth and the values reported are the mean of three replicates. Error bars indicate the standard deviation.
References


